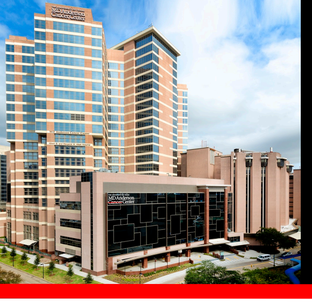

Molecular Adaptations of Mouse Lung Endothelial Cells Exposed to Different Durations of Laminar Shear Stress and Disturbed Flow

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Background

Endothelial cells (ECs) are constantly exposed to shear stress, the tangential frictional force acting on the vessels due to blood flow. Shear stress may be divided into two main types, depending on the intensity and flow patterns: laminar shear stress (LSS), characterized by unidirectional, regular flow patterns found in straight regions of vessels, and disturbed flow (DF), which has a low and oscillatory flow pattern found in curved and bifurcated sites of vessels as well as in tumor vasculature.

Whereas LSS promotes healthy vasculature by stabilizing the EC layer, DF impairs EC function by doing the opposite. ECs sense these mechanical forces by a variety of mechanosensors and transducers. Among them, sphingosine 1-phosphate receptor 1 and 2 (S1PR1, S1PR2) are two G protein-coupled receptors involved in EC stabilization and function. Whereas S1PR1 activity leads to increased vascular barrier function, S1PR2 impairs vessel permeability and integrity, and both are differentially modulated by LSS and DF.

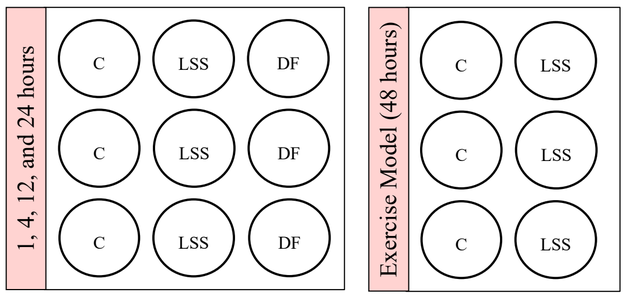
Recently, a potential link between S1P receptors and mitochondrial homeostasis has been suggested. The modulation of S1PR1 and S1PR2 affects mitochondrial morphology and function in different cell types, protecting from cell injury and oxidative damage. Mitochondrial respiration is also enhanced by exercise-induced LSS, resulting in improved endothelial cell function. However, the relationship between S1PR1, S1PR2 and mitochondria function in ECs in response to shear stress have never been addressed.

Project Aim

1	Characterize the molecular signature of mouse lung ECs (mLECs) in response to LSS and DF at different durations.
2	Mimicking, *in vitro,* the conditions that may be found in vessels under exercise training (LSS) or in tumor vasculature (DF).

Experimental Outline

Isolation of mLECs	1. Lung Extraction and Digestion 2. CD31 Microbead Separation 3. Cultivation in EC Specific Medium
Shear Stress Exposure	1. LSS (20 dyn/cm^2) and DF (3 dyn/cm^2) applied for 1, 4, 12, or 24 hours 2. To mimic exercise: low LSS (5 dyn/cm^2) for 2 days with 1 hour of high LSS (20 dyn/cm^2) twice a day



Gene Expression Analysis	Real time PCR analysis to analyze: • Shear stress-induced proteins (KLF2 and KLF4) • Inflammation-related proteins (ICAM-1 and VCAM-1) • S1PR1 and S1PR2 • Mitochondrial function indicators (Cytochrome C and SDHA)

Expected Results

Target Gene	Laminar Shear Stress	Disturbed Flow
Shear stress-induced proteins (KLF2 & KLF4)	Increase	Decrease
Mitochondrial Function	Increase	Decrease
S1PR1	Increase	Decrease
S1PR2	Decrease	Increase
Inflammation-related proteins (ICAM-1 & VCAM-1)	Decrease	Increase

Endothelial cells were successfully cultured using novel protocol

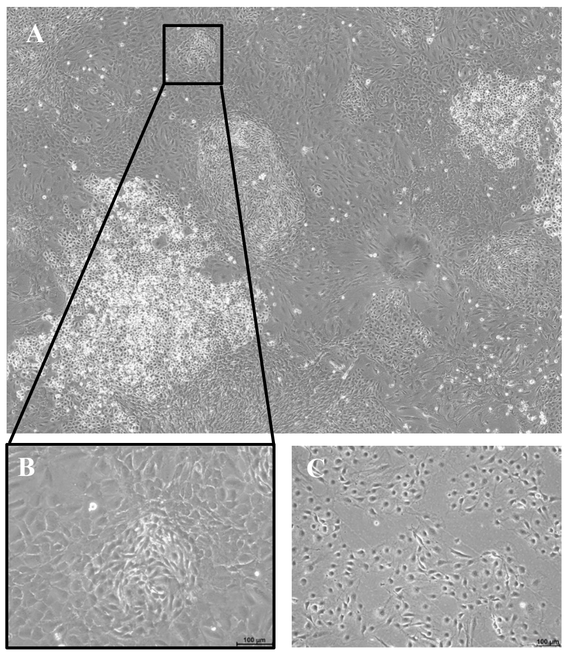


Figure 1. Phase contrast pictures of (A) cell population plated after lung digestion (B) a cluster of (C) ECs after CD31 microbead isolation at passage 3 before shear stress exposure. All the images were taken at a 10X magnification.

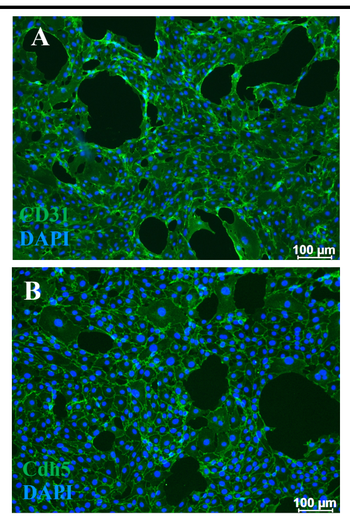


Figure 2. Fluorescence microscopy pictures of ECs stained for (A) CD31 and (B) Cdh5 Cells were stained for DAPI (Blue) and CD31 and Cdh5 (Green). All the images were taken at a 10X magnification. Most of the cells were positive for both the EC-specific indicators

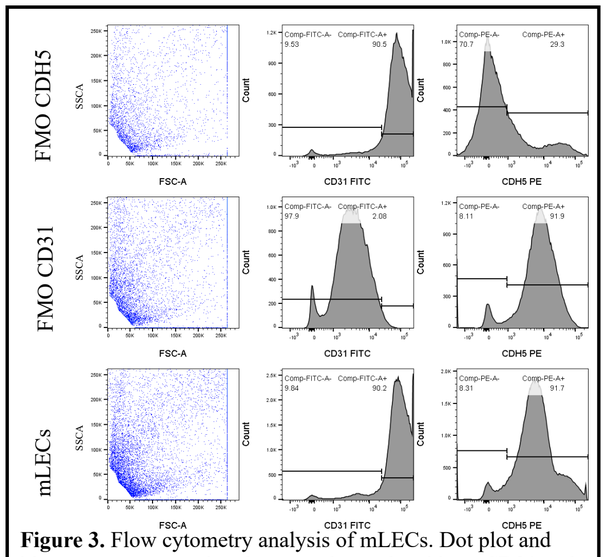


Figure 3. Flow cytometry analysis of mLECs. Dot plot and histograms representing the control sample without CD31 and Cdh5 antibody (A and B respectively) and (C) mLECs stained for both CD31 and Cdh5. The positive expression of of CD31 (90.2%) and Cdh5 (91.7%) characterized these cells as endothelial.

Target genes followed predicted trends in a shearing time-dependent manner

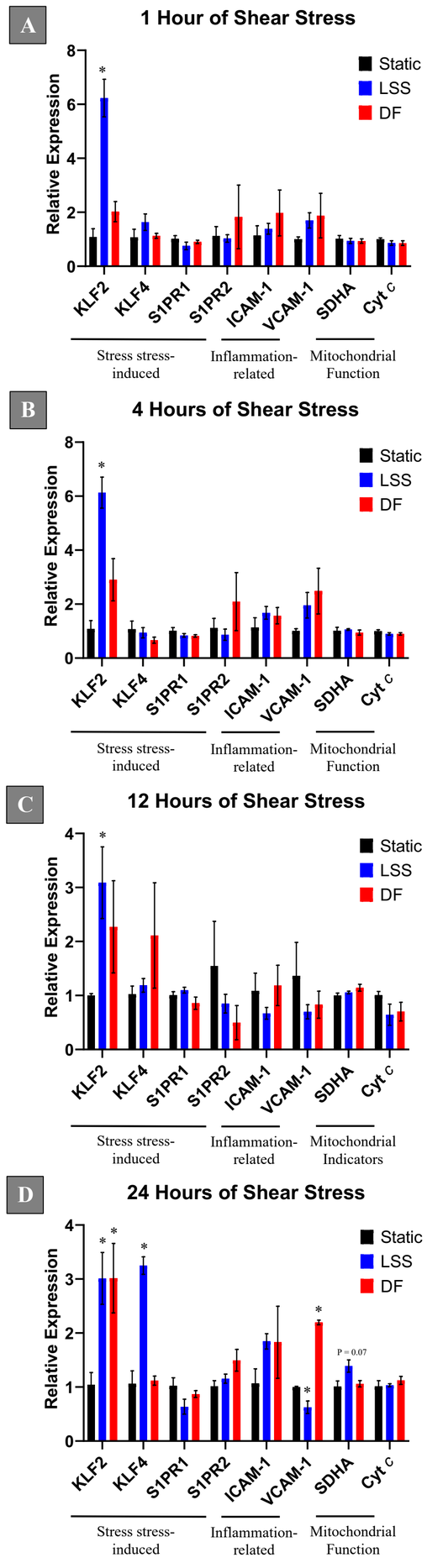


Figure 4. Relative gene expression (mean ± SEM) for shear stress-induced proteins, mitochondrial function indicators, S1PR1, S1PR2, and inflammation-related proteins after 1 hour (A), 4 hours (B), 12 hours (C), and 24 hours (D) of shear stress exposure. mLECs were kept at a static condition or exposed to LSS or DF. The experiment was performed in triplicate for each condition. Significant values were observed at $p < 0.05$.

Gene expression changes induced by exercise model shearing reflected *in vivo* exercise

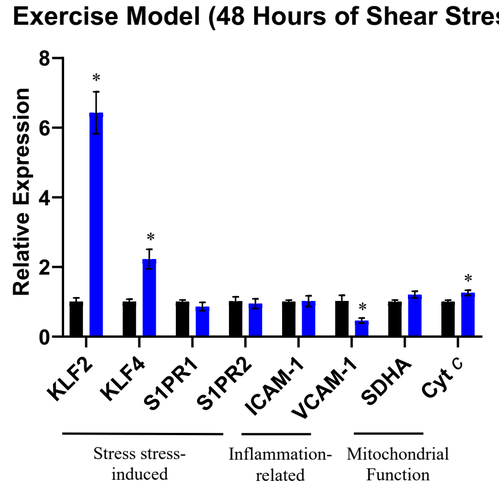


Figure 5. Relative gene expression (mean ± SEM) for shear stress-induced proteins, mitochondrial function indicators, S1PR1, S1PR2, and inflammation-related proteins after 48 hours low LSS with intermittent 1 hour of high LSS twice a day. mLECs were kept at a static condition or exposed to LSS. The experiment was performed in triplicate for each condition. Significant values were observed at $p < 0.05$.

Conclusion

- Endothelial cells were successfully isolated using our protocol and can be applied to future experiments pertaining to vascular function
- LSS leads to increase in KLF2, KLF4, and Cytochrome C and decrease in VCAM-1
- DF leads to increases in KLF2 and VCAM-1
- As shearing duration is increased, more *in vivo* exercise conditions are mimicked.
- Our 24-hour showed pronounced effects when exposed to shear stress.
- The intermittent shearing included in our 48-hour exercise model mimicked *in vivo* exercise.

Future Studies

- Attempt isolation of endothelial cells from tumors and use the proposed exercise model to mimic in vivo exercise effect
- Test for additional mitochondrial related genes (eg. PGC-1α)

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